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Study of formation of jarosite mediated by thiobacillus ferrooxidans in 9K medium

Liu Jin-yan, Tao Xiu-xiang*, Cai Pei

School of Chemical Engineering and Technology, China University of Mining & Technology, Xuzhou 221008, China

Abstract

Thiobacillus ferrooxidans, occurring extensively in mine districts, have important effects on the oxidation of metal sulphide and the formation of jarosite. In the coal bio-desulphurization, jarosite mediated by microorganisms decreases the bacterial absorbability of nutrients, occupies the available sites of coal surfaces, ultimately results in residual sulfur, which cannot be removed from coal. The thiobacillus ferrooxidans cultivation conditions with minimal jarosite in 9K medium by varying the initial pH, the initial Fe\textsuperscript{2+} concentration and the applied potential were studied and determined. Experimental results show that the optimal combination giving the minimal jarosite precipitates (3.73 g/L) and the good growing activity of bacteria is the initial pH of 1.6-1.7 with the Fe\textsuperscript{2+} concentration of 9-10 g/L and the applied potential of -0.5 V for 7 hours. The results will be of significant importance for the further research on the bacterial cultivation and coal desulphurization.

Keywords: jarosite; thiobacillus ferrooxidans; 9K medium; applied potential

1. Introduction

Thermophilic microbes, such as thiobacillus ferrooxidans, occurring extensively in metal sulphide ore districts have an important effect on oxidation of metal sulphide and formation of secondary mineral-jarosite. Thiobacillus ferrooxidans (T.f for short) are gram-negative chemolithotrophic bacteria, characterized by rods with rounded ends, 1.0-2.0 µm long and 0.5-0.8 µm wide, occurring singly or in pairs, rarely in short chains. They are also known to be motile by means of a single polar flagellum. T.f, with CO\textsubscript{2} as the carbon source and NH\textsubscript{4}\textsuperscript{+} as the nitrogen source, can obtain energy from the oxidation of different inorganic compounds, the most common of which is ferrous sulfate. The oxidation is catalyzed by a series of enzymes such as Fe\textsuperscript{2+} cytochrome C\textsubscript{552} oxidoreductase, cytochrome oxidase, sulfur oxidase and so on [1]. T.f have been widely utilized in the processes of bioleaching, desulphurization of sour gases, treatment of acid mine drainage and the desulphurization of coal [2-4]. The dark yellow precipitates produced during the ferrous oxidation by T.f have been proved the restriction to the oxidation of T.f and the efficiency of coal desulphurization by other authors [5].
1.1. Jarosite formation

T. f commonly grow on 9K liquid medium, containing: (NH₄)₂SO₄ 3.0 g/L, MgSO₄·7H₂O 0.5 g/L, K₂HPO₄ 0.5 g/L, KCl 0.1 g/L, Ca(NO₃)₂ 0.01 g/L, FeSO₄·7H₂O 44.78 g/L. The overall oxidation of ferrous ions is catalyzed by T. f via reaction (1):

$$4\text{Fe}^{2+} + \text{O}_2 + 4\text{H}^+ \xrightarrow{T. f} 4\text{Fe}^{3+} + 2\text{H}_2\text{O}$$

(1)

Because of the consumption of H⁺ in reaction (1), the pH of the liquid media initially increases. However, the pH increase is counteracted by the hydrolysis of Fe³⁺ [6]. Therefore, the pH of the liquid system has effect on the extent of the oxidation and hydrolysis reactions. Furthermore, a competitive reaction with the hydrolysis reaction occurs giving products of Fe⁶⁺ hydroxysulphates with the formula MFe₃(SO₄)₂(OH)₆, where M= K⁺, Na⁺, NH₄⁺, Ag⁺ or H₃O⁺. The hydroxysulphate precipitates are known as jarosites. Since the 9K medium contains a high concentration of NH₄⁺, the jarosites chiefly produced are ammoniojarosites with the formula NH₄Fe₃(SO₄)₂(OH)₆. It follows the following chemical equation:

$$3\text{Fe}^{3+} + \text{NH}_4^+ + 2\text{SO}_4^{2-} + 6\text{H}_2\text{O} \rightarrow \text{NH}_4\text{Fe}_3(\text{SO}_4)_2(\text{OH})_6 + 6\text{H}^+$$

(2)

If the oxidizing rate of Fe²⁺ is defined as:

$$\frac{d[\text{Fe}^{2+}]}{dt} = -V_{\text{Fe}^{2+}}$$

(3)

The producing rate of precipitates is defined via Fe³⁺ consumption of reaction (2):

$$\frac{d[\text{Fe}^{\text{ppt}}]}{dt} = -V_{\text{ppt}}$$

(4)

Then, the mass balances for Fe³⁺ and H⁺ in solution are given by the equations:

$$\text{Fe}^{3+}: \frac{d[\text{Fe}^{3+}]}{dt} = V_{\text{Fe}^{3+}} - V_{\text{ppt}}$$

(5)

$$\text{H}^+: \frac{d[\text{H}^+]}{dt} = -V_{\text{Fe}^{3+}} + 2V_{\text{ppt}}$$

(6)

Therefore the producing rate of precipitates $V_{\text{ppt}}$ can be expressed as:

$$V_{\text{ppt}} = \frac{d[\text{H}^+]}{dt} + \frac{d[\text{Fe}^{3+}]}{dt}$$

(7)

1.2. Effect of jarosite on coal desulphurization

Jarosite, a kind of crystal precipitate, attaching on the surface of the bio-oxidized coal particles results in a compact thin layer of membrane, which may hinder the transfer of substrates and metabolites and decrease bacterial absorbability of nutrients; it can also occupy the available sites of coal surfaces to prevent bacteria from adsorption on coals. Furthermore, it results in residual sulfur, which cannot be removed from coal.

There is a growing awareness of the jarosite disadvantages in coal bio-desulphurization. Therefore, several studies have been conducted to determine the influences of jarosite formation in bioreactions. The experiments have concluded that Fe³⁺ competitively inhibit Fe²⁺ oxidation by T.f and the inhibitory effect can be reduced by increasing cell concentration; Moreover, the pH and temperature have significant effects on the oxidation kinetics of Fe²⁺ by T.f; the amount of jarosite can be decreased by moderately reducing the NH₄⁺ concentration in medium [6-8]. However, no articles published have dealt with controlling the amount of jarosite formation by applied potential, which can directly influence the bacteria growth and the concentration of Fe²⁺/Fe³⁺.
In accordance with formation of jarosite, the main goal of this study is to determining the T.f cultivation conditions with minimal jarosite precipitates in 9K medium by varying the initial pH, the concentration of Fe\(^{2+}\) and the applied potential. The results will be of significant importance for the further research on the coal biodesulphurization.

2. Materials and methods

2.1. Bacteria cultivation

*T.f* used in this work have been isolated from the acid mine drainage of Zibo Coal Mining Administration in Shandong Province. Growing on 9K medium, the bacteria were incubated in 250 mL Erlenmeyer flask containing 135 mL of the medium and 10% (v/v) inoculums, on a rotary shaker at 110 rpm at constant temperature of 28 °C. The initial pH of the culture was adjusted using 1 N H\(_2\)SO\(_4\). Every experiment was set up in duplicate.

2.2. Experimental equipments and methods

The acidity of the cultural suspensions was monitored with the No. S-3CA model pH meter. The concentration of free bacteria in solution was determined by direct counting using a Thoma chamber of 0.1mm depth and 0.0025 mm\(^2\) areas with the optical X5Z-H microscope and the WV-CP240/G micro-vidicon. The Fe\(^{2+}\) concentration was determined by a titrimetric method with potassium permanganate as the titrant. The Fe\(^{3+}\) concentration in the resulting solution was measured spectrophotometrically using 5-Sulfosalicylic acid as an indicator [9]. The applied potential was provided by the CHI601B electrochemical analyzer with a three-electrode system including a platinum disk working electrode, a platinum plate counter electrode and an Ag/AgCl reference electrode. Jarosite precipitates were identified by the means of Nicolet-560 Fourier transform infrared spectroscopy. The amount of jarosite precipitates was measured through the loss of total iron concentration in the 9K medium.

All the chemicals used in the work were of analytical grade, and all the experimental results were identified by averaging three groups of numbers.

3. Results and discussions

3.1. Identification of jarosites

The jarosites, formed in the 9K medium with pH of 2.00 through the bacteria, were collected and then air dried naturally. Those jarosites were identified by the means of Fourier transform infrared spectroscopy (FTIR). Fig. 1 indicated that: the strong absorption peak in the region of 2900-3700 cm\(^{-1}\) was ascribed to the O-H stretching; the absorption peak of 1425 cm\(^{-1}\) were attributed to the NH\(_4^+\) stretching the \(\nu_4\), \(\nu_3\), \(\nu_2\) and \(\nu_1\) vibration of SO\(_4^{2-}\) were represented in the wavenumbers of 628, 1194, 1085, and 1004 cm\(^{-1}\), respectively; the vibration peaks of FeO\(_6\) octahedron were observed in the wavenumbers of 511 cm\(^{-1}\) and 473 cm\(^{-1}\). Consequently, the above results prove the precipitates bio-mediated to be amino jarosites.

3.2. Influence of initial pH

The shift of pH results from the balance between the simultaneous consumption of H\(^+\) in the oxidation of Fe\(^{2+}\) and the release of H\(^+\) related to Fe\(^{3+}\) precipitates, hence the amount of jarosite and the growing rate of T.f can be controlled by pH adjusting.

The inhibition of bacterial oxidation activity at pH values over 2.3 is partially linked to the formation of a layer of precipitates on bacteria, which hinders the diffusion of H\(^+\). At pH below 1.5, the decrease in bio-oxidation is related to the inhibition of bacterial activity by acidity. Thus the author tried to analyze the relationship of Fe\(^{2+}\) transformation efficiency, Fe\(^{3+}\) yield rate and the jarosite precipitates by adjusting the initial pH as 1.58, 1.63, 1.69, 1.73, 1.79 and 1.99, respectively.
Continuously incubated at the initial pH 2.0 of 9K culture, T.f expressed the highest Fe\(^{2+}\) oxidation ability, whereas after the lag phase of two days, it grew well in the acidy situation of pH 1.6-1.7. Fig. 2 indicates that at the initial pH of 1.63, Fe\(^{2+}\) transformation efficiency ascends rapidly, and after 4.5 days Fe\(^{2+}\) in the situations of pH=1.99, 1.63, 1.68 are biooxidated completely. In contrast, Fe\(^{2+}\) transformation efficiency of 120 h in other pH values is lower than 90%.
According to Fig. 2 and Fig. 3, in the first 40 hours, the lower Fe\(^{2+}\) transformation efficiency and the less Fe\(^{3+}\) yields were the representation of slow metabolize of T.f in lag phase; then with taking full advantage of Fe\(^{2+}\) as energy sources, the bacteria metabolized lots of Fe\(^{3+}\); after that, the formation of jarosites according to the reaction (2) led to the decrease of Fe\(^{3+}\) concentration. In the cultural situation of pH 1.63 and 1.68, the bacteria gained much faster yield rate of Fe\(^{3+}\). Fig. 4 showed the relationship between jarosite mass and pH values. With pH increasing, the jarosite amounts increased, too. The optimal pH were 1.6-1.7, in which conditions T.f produced the less jarosite while still maintaining higher growing rate so as to accelerate the coal biodesulphurization efficiency.

3.3. Influence of initial Fe\(^{2+}\) concentration

The oxidation of Fe\(^{2+}\) to Fe\(^{3+}\) represented the metabolized activity of T.f for Fe\(^{2+}\) was the energy resource of T.f [10]. As expression (7) showed before, the producing rate of jarosite in the medium depended on the pH and the concentration of Fe\(^{3+}\) which linked directly with the initial Fe\(^{2+}\) concentration. In the condition of pH=1.6-1.7, the results of T.f biooxidation at different initial Fe\(^{2+}\) concentrations were presented in Fig. 5.

As the initial Fe\(^{2+}\) concentration of 10 g/L, which was consistent with that of 9K medium, T.f showed the vigorous metabolism and after two days the bacteria population reached 10\(^8\)/mL. K.Shihari et al have suggested that the lag phase of T.f growth can be prolonged by the higher Fe\(^{3+}\) concentration [11]. In the lower Fe\(^{2+}\) concentration (5 g/L), T.f growth experienced a short lag phase and an innutrient exponential phase and then a slow decline phase with the most bacteria population of only 5\times10\(^7\)/mL. While inoculated in the initial Fe\(^{2+}\)concentration of 20 g/L, 30 g/L and 50 g/L respectively, T.f revealed a prolonged lag phase and a inhibited growth to some extent, for the higher initial Fe\(^{2+}\) concentration after the biooxidation and air-oxidation caused the faster increase of Fe\(^{3+}\) concentration.

After seven days' cultivation, the amount of jarosite and the oxidizing efficiency of Fe\(^{2+}\) were measured and listed in Table 1. The consumption of Fe\(^{2+}\) with the initial Fe\(^{2+}\) concentration of 20 g/L, 30 g/L and 50 g/L was calculated to be 16.64 g/L, 16.74 g/L and 16.25 g/L respectively. That is, in the condition of pH=1.6-1.7, Fe\(^{2+}\) biooxidation can be restricted totally and jarosite precipitates were formed massively when the initial Fe\(^{2+}\) concentration higher than 17 g/L. So in order to maintain the energy supply for the bacteria metabolism as well as the moderate Fe\(^{3+}\) concentration, the optimum concentration of initial Fe\(^{2+}\) were ranged from 9 g/L to 10 g/L. The result was in accordance with the magnetosome high expression under 150-200 mmol/L FeSO\(_4\)-7H\(_2\)O [12].

Table 1. Jarosite amount and Fe\(^{2+}\) oxidizing rate in different initial [Fe\(^{2+}\)]

<table>
<thead>
<tr>
<th>Initial[Fe(^{2+})] (g/L)</th>
<th>Fe(^{2+}) oxidizing efficiency (%)</th>
<th>Jarosite amount (g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>99.9</td>
<td>1.73</td>
</tr>
<tr>
<td>10</td>
<td>98.5</td>
<td>6.07</td>
</tr>
<tr>
<td>20</td>
<td>83.2</td>
<td>10.92</td>
</tr>
<tr>
<td>30</td>
<td>55.8</td>
<td>16.57</td>
</tr>
<tr>
<td>50</td>
<td>32.5</td>
<td>21.08</td>
</tr>
</tbody>
</table>

3.4. Influence of applied potential

Certain applied electric field to T.f can optimize the permeability of cell membranes and accelerate the metabolic rate. Previous studies have demonstrated that oxygen and intermediate-H\(_2\)O\(_2\) produced by the electrolysis of the acidic bacteria solution under positive potential interfere with the metabolism of T.f cells; the positive potential also leads to the shortage of energy supply for Fe\(^{3+}\) is more stable than Fe\(^{2+}\) in medium[13-15]. Whereas, negative potential is beneficial to the reduction of Fe\(^{3+}\) to Fe\(^{2+}\), which can guarantee the energy need of bacteria, thereby enhancing the growing activity and lessening the formation of jarosite precipitates [16]. The influence of applied negative potential to the bacteria growth was discussed in the study.

In Fig. 6, two pairs of redox peaks in the cyclic voltammetry curves represented the redox reaction of Fe\(^{2+}\)-Fe\(^{3+}\) and Fe\(^0\)-Fe\(^{2+}\) respectively, as expressions shown below:
\[
Fe^{3+} + e = Fe^{2+} \quad \phi^0 = 0.783 \text{V} \\
Fe^{2+} + 2e = Fe \quad \phi^0 = -0.447 \text{V}
\]

Comparing with the two standard electrode potentials, the Fe\(^{3+}\) is easily reduced to Fe\(^{2+}\) and Fe easily oxidized to Fe\(^{3+}\). After different negative potentials were applied to the bacteria solution for 7 hours, the cyclic voltammetry curves were made to show that, the effect of -0.5 V potential to the Fe\(^{3+}\) reduction was obviously better than -0.3 V or -0.7 V, for the peak current was maximum \((i=5.542 \times 10^{-4} \text{A})\).

Catalyzed for 7 hours in -0.5 V applied potential, T.f was then cultivated in the rotary shaker. The variations of Fe\(^{2+}\) and Fe\(^{3+}\) concentrations were illustrated in Fig. 7. There was an overall downward trend of Fe\(^{2+}\) concentration and upward trend of Fe\(^{3+}\) concentration. In the first 7 hours, the undulation of Fe\(^{2+}\) and Fe\(^{3+}\) concentrations was due to the T.f catalysis of Fe\(^{2+}\) to Fe\(^{3+}\) and the electrochemical reduction of Fe\(^{3+}\) to Fe\(^{2+}\). In the subsequent two days, a sharp decline of Fe\(^{2+}\) concentration was observed. It was because T.f domesticated by the negative potential showed a vigorous growing and reproducing activity. On the second day of cultivation, T.f growth came to the exponential phase. And at the end of the third day, most of Fe\(^{3+}\) was oxidized by T.f. In the process of bio-reaction, the amount of total Fe changed rarely, and the whole jarosite precipitates generated was only 3.73 g/L. Compared with the value 6.01 g/L in the non-potential condition, the amount of jarosite had decreased by 38.6%.

4. Conclusions

1) The dark yellow precipitates produced during the Fe\(^{2+}\) oxidation by T.f were identified to be ammoniojarosites by the means of Fourier transform infrared spectroscopy.

2) In the condition of initial pH=1.6-1.7, T.f maintained the higher Fe\(^{2+}\) oxidizing efficiency while minimal amount of jarosite was mediated by T.f in the 9K medium.

3) Under the lower initial acidity of medium (pH=1.6-1.7), when the initial Fe\(^{2+}\) concentration higher than 17 g/L, Fe\(^{3+}\) biooxidation can be restricted totally and jarosite precipitates were formed massively.

4) The optimal combination giving the minimal jarosite precipitates(3.73 g/L) and the vigorous growing activity of bacteria is the initial pH of 1.6-1.7 with the Fe\(^{2+}\) concentration of 9 g/L-10 g/L and the applied potential of -0.5 V for 7 hours.
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References